1. Introduction

The international Genetically Engineered Machine (iGEM) competition is the largest synthetic biology competition for collegiate students. It aims to combine and create novel biological (genetic) parts, which can be incorporated in an organism, thereby equipping it with new functions. This year our work will be presented at the Giant Jamboree to the international scientific community in Boston in October 2016. An iGEM project consists of lab work, modelling, policy & practice and public outreach with results being documented on a wiki page.

This year the team will try to establish lasing in bacteria and will research the possibilities of making biological lenses. Imaging of cells is one of the most essential parts of research of biological systems. Imaging of cells leads to more insights in cells and cellular systems. Knowing what happens in a cell is of essential importance when trying to understand life at the smallest scale or when fighting diseases. However, most imaging techniques are fluorescence based and therefore have a too low resolution to form a proper image due to limited amount of photons is emitted. Increasing resolution is often achieved by using high-tech microscopes, but this is a very expensive solution. We are trying to tackle this issue by increasing the resolution of the light emitted by the cell by establishment of lasing in the cell. We will achieve lasing by expressing reflective agents such as polysilicate and PHB outside and inside the cell, respectively, so the photons emitted by the fluorescent proteins will not escape the cell but resonate until they gain a certain energy. This will lead to light with a higher, which will improve the resolution of imaging.

Besides the lasing project, we will also be working on developing a biological microlens that will be able to capture more light and act as a biological and environmentally friendly alternative to the currently used microlenses. We will make cells express a gene that will make the cells perfectly round and a gene expressing a protein that can cover the cell in polysilicate. This will result in a round cell that can act like a lens. A possible application of these biolenses is biolens arrays, that can be used in small, lightweight cameras, solar panels and 3D screens.

2. Regulations

The materials will be handled cautiously wearing appropriate gloves and always working according good safety and laboratory practices. ML-1 safety requirements will be followed, and all the team members are obliged to follow four mandatory safety tests (Biological safety ML-1, General Safety TNW Zuid, Lab safety and basic Laser safety) before entering any lab.

The project will be done according the **iG04-076 permit** from the Department of Bionanoscience.

The work described is according to the Dutch GMO laws, under **'inschalingsartikel 5.2i inperkingsniveau MLI'.** For requirements, see appendix 1.

3. Biological safety

The aim of the 2016 TU Delft iGEM team is to make cells with an improved light emission, which is obtained by an intracellular lasing effect and a cell that can function as a lens, using the microorganism *Escherichia coli.* The *E. coli* strains used are non-infectious and non-pathogenic and are scaled under biosafety level 1, see table below.

Host species	Strains	Biosafety level
Escherichia coli B	BL21	1
Escherichia coli K12	TOP10	1

All materials will be provided by the department of Bionanoscience. Before working with biological material, all students will have taken a Biological safety ML-1 course. Students that have no Life Science & Technology background or have no experience in working with microorganisms will be under guidance by supervisor Esengül Yildirim for at least four weeks in order to learn good VMT practices.

The genes would be carried by the standard plasmids according to the iGEM rules (BioBrick Plasmid Backbone), and they would be introduced into *E. coli* via DNA transformation. The table below shows all inserts that are going to be used for the project, their native organism and function:

Insert name	Donor organism	Biosafety level	Function
GFP mut3B	Derived from *Aequorea Victoria* [1]	For just protein: 1	Enhanced green fluorescent protein
mKate	Derived from *Entacmaea quadricolor* [2]	For just protein: 1	Red fluorescent protein
mVenus	Derived from *Aequorea Victoria* [3]	For just protein: 1	Yellow fluorescent protein
mCerulean	Derived from *Aequorea Victoria* [4]	For just protein: 1	Blue fluorescent protein
silicaa-g	*Suberites domuncula* [5]	For just protein: 1	Silicatein protein, synthesis of polysilicate
Silicatein-alpha	*Tethya aurantia* [6]	For just protein: 1	Silicatein protein, synthesis of polysilicate
inaK	*Pseudomonas syringae* [7]	1	Transmembrane domain of ice nucleation protein
OmpA	*Escherichia coli K12* [8]	1	Transmembrane domain of a variety of membrane proteins
BolA	*Escherichia coli K12* [9]	1	Transcriptional regulator in general stress response. Changes cell morphology to circular.
SulA	*Escherichia coli K12* [10]	1	Inhibitor of cell division. Changes cell morphology to filamentous
PHA operon (phaC, phaA, phaB)	*Ralstonia eutropha* [11]	1	Synthesis of intracellular polyhydroxyalkanoate (PHA)
phaP	*Ralstonia eutropha* [11]	1	Enlargement of PHA granules

The two silicatein genes will be separately fused to the two transmembrane enzymes, in order to express membrane-anchored silicatein. The fluorescent proteins will be expressed in combination with either the (membrane-anchored) silicatein or the PHB to establish intracellular lasing. The bolA gene will be co-expressed with silicatein to make a biological lens. The bolA and sulA genes will be expressed in the intracellular lasers to determine the effect of cell shape on lasing. The plasmids used are shown in appendix 2.

The main experiments that are going to be performed for introducing the new genes, and the assays for testing the outcome of the experimentations, are:

- Transformation
- Restriction with enzymes
- Purification of nucleic acids
- Precipitation of nucleic acids
- Ligation
- PCR
- DNA gels
- Microscopy (confocal & SEM)
- Spectroscopy
- Interferometry

4. Chemical safety

Before working with any chemicals, all students will have taken the Lab safety course. This project will not make use of dangerous chemical compounds. For genetic engineering we will use standard genetic engineering reactants, buffers and media. Furthermore, we will use the following chemicals:

Chemical	Function	Safety and disposal
Silicic acid hydrate	Substrate for silicatein	See appendix 3
Tetraethyl orthosilicate (TEOS)	Substrate for silicatein	See appendix 4
Rhodamine 123	Visualisation of polysilicate	See appendix 5
Silicate Test (Merck 100857)	Determine enzymatic activity silicatein	See appendix 6
Ethidium Bromide	Gel staining	See appendix 7
Lithium hexafluorostannate	Tin oxide layer around the cells	See appendix 8

5. Laser safety

Before working with any setups, all students will have taken the basic laser safety course of the Department of Bionanoscience. The lasing experiments will be conducted in a confocal microscope, under guidance. If we are going to use more advanced setups, an advanced laser safety test will be taken. The experiments involving lasers will be supervised by someone with significant experience with lasers.

6. References

[1] Cormack, B.P., Valdivia, R.H., and S. Falkow. FACS-optimized mutants of green fluorescent protein (GFP). Gene 173: 33-38 (1996).

[2] Shcherbo, Dmitry, et al. "Bright far-red fluorescent protein for whole-body imaging." Nature methods 4.9 (2007): 741-746.

[3] Nagai, Takeharu, et al. "A variant of yellow fluorescent protein with fast and efficient maturation for cell-biological applications." Nature biotechnology 20.1 (2002): 87-90.

[4] Rizzo, Mark A., et al. "An improved cyan fluorescent protein variant useful for FRET." Nature biotechnology 22.4 (2004): 445-449.

[5] Müller, Werner EG, et al. "Silicateins, the major biosilica forming enzymes present in demosponges: protein analysis and phylogenetic relationship." Gene 395.1 (2007): 62-71.

[6] Shimizu, Katsuhiko, et al. "Silicatein α: cathepsin L-like protein in sponge biosilica." Proceedings of the National Academy of Sciences 95.11 (1998): 6234-6238.

[7] Jung, Heung-Chae, Jean-Michel Lebeaultz, and Jae-Gu Pan. "Surface display of Zymomonas mobilis levansucrase by using the ice-nucleation." Nature biotechnology 16 (1998).

[8] Francisco, Joseph A., Charles F. Earhart, and George Georgiou. "Transport and anchoring of beta-lactamase to the external surface of Escherichia coli." Proceedings of the National Academy of Sciences 89.7 (1992): 2713-2717.

[9] Aldea, M., et al. "Identification, cloning, and expression of bolA, an ftsZ-dependent morphogene of Escherichia coli." Journal of bacteriology 170.11 (1988): 5169-5176.

[10] Siddiqui, Roman A., et al. "The analysis of cell division and cell wall synthesis genes reveals mutationally inactivated ftsQ and mraY in a protoplast-type L-form of Escherichia coli." FEMS microbiology letters 258.2 (2006): 305-311.

[11] Pohlmann, Anne, et al. "Genome sequence of the bioplastic-producing “Knallgas” bacterium Ralstonia eutropha H16." Nature biotechnology 24.10 (2006): 1257-1262.

Appendix 1 - inschalingsartikel 5.2i inperkingsniveau MLI

5.2 Activities with a genetically modified micro-organism whose host is listed in List A1 in Appendix 2 (E.coli die jullie gebruiken staat hierin) and whose vector is either listed in List A2 in Appendix 2 or meets certain criteria, as follows:

i. the size of the vector is known;

ii. a vector map or description is available, including all the vector's composite parts and their relative positions;

iii. the functions and origins of the composite parts are known;

iv. the oris present in the vector are known;

v. the composite parts are not members of the group of insertions listed in Appendix 2, List A3; (vertaling beschrijving; schadelijke genproducten zoals toxines etc.) if they are, then those parts should be regarded as donor sequences;

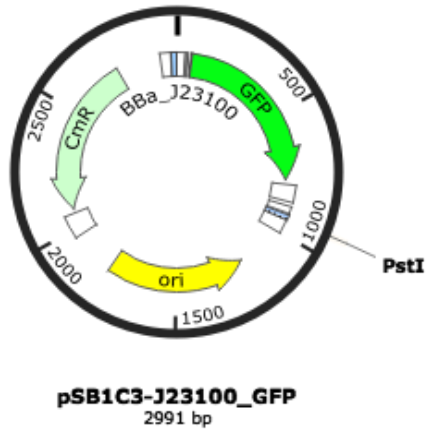
vi. the vector contains no viral sequences from viruses hosted by higher eukaryotes, which might enable the vector to act as a viral vector; if it does, then those sequences should be regarded as donor sequences.

Activities in which characterised donor sequences are used:

i. The sequence contains no genetic information that codes for a harmful gene product. Classification: ML-I.

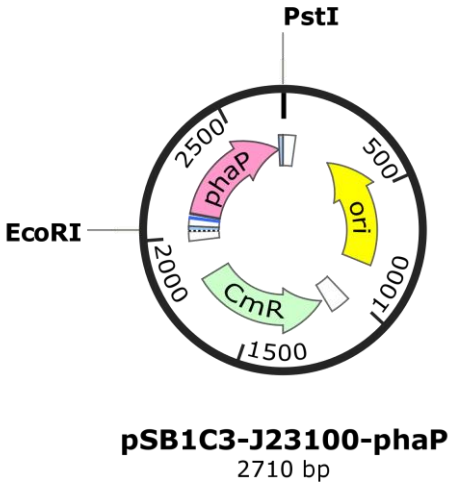
Appendix 2 – Vectors used

GFP insert:



Promoter: BBa_J23100 (combinatorial *E. coli B* constitutive promoter iGEM)

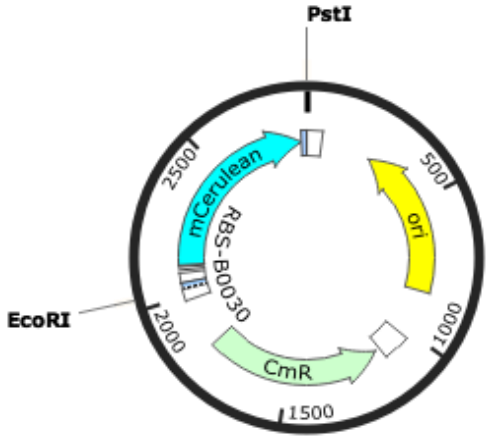
Terminator: T1 from E. coli B



Promoters: BBa_J23113, BBa_J23117, BBa_J23105, BBa_J23108, BBa_J23100

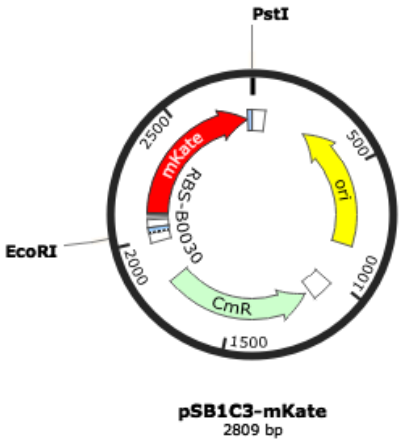
(combinatorial *E. coli B* promoter iGEM of different strengths)

Terminator: T1 from E. coli B



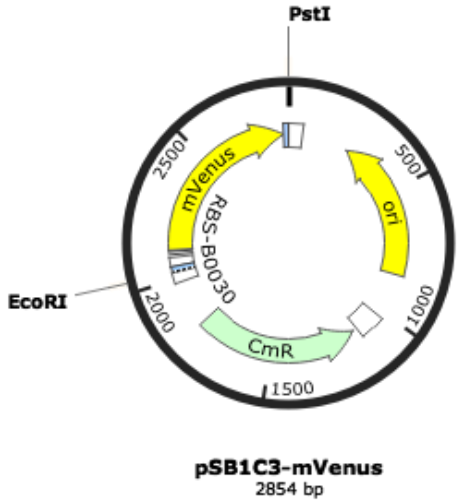
Promoter: BBa_J23100 (combinatorial *E. coli B* constitutive promoter iGEM)

Terminator: T1 from E. coli B



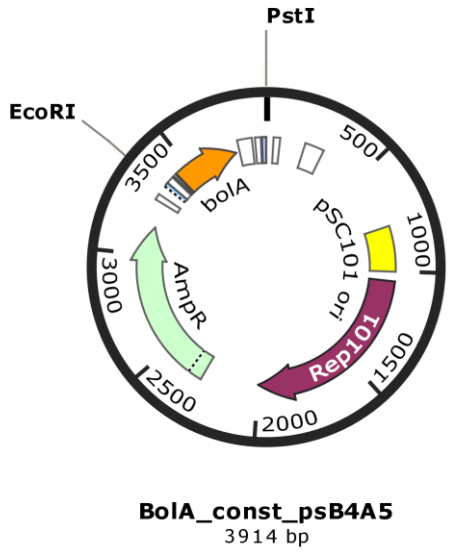
Promoter: BBa_J23100 (combinatorial *E. coli B* constitutive promoter iGEM)

Terminator: T1 from E. coli B



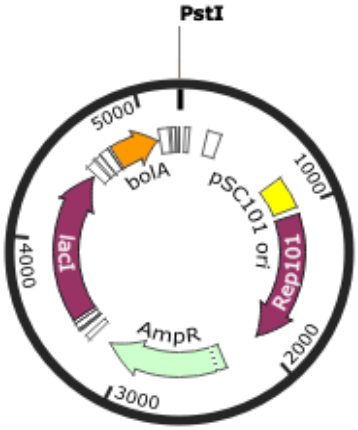
Promoter: BBa_J23100 (combinatorial *E. coli B* constitutive promoter iGEM)

Terminator: T1 from E. coli B



Promoter: BBa_J23100 (combinatorial *E. coli B* constitutive promoter iGEM)

Terminator: T1 from E. coli B



pSB4A5-lacI_bolAind
5226 bp

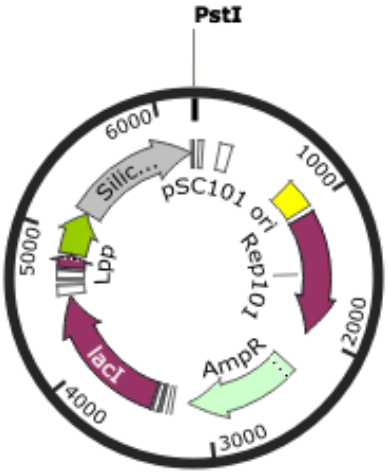
Promoter: *LacI* (*E. coli B*)

Terminator: T1 from E. coli B

pSB4A5-lacI_INPNC_Sdum
6184 bp

Promoter: *LacI* (*E. coli B*)

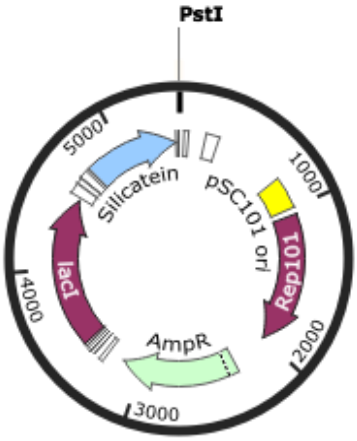
Terminator: T1 from E. coli B



pSB4A5-lacI_OmpA_Taur
6223 bp

Promoter: *LacI* (*E. coli B*)

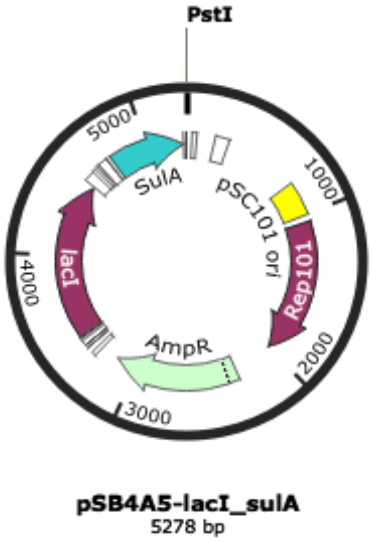
Terminator: T1 from E. coli B



pSB4A5-lacI_Sdum
5428 bp

Promoter: *LacI* (*E. coli B*)

Terminator: T1 from E. coli B



Promoter: *LacI* (*E. coli B*)

Terminator: T1 from E. coli B

Appendix 3: Mini MSDS Silicic acid hydrate

Retrieved from the Chemwatch server

NON-HAZARDOUS CHEMICAL. NON-DANGEROUS GOODS. According to the WHS Regulations and the ADG Code.

SILICIC ACID

INGREDIENTS	CAS NO	%	8HR OEL
silicic acid	1343-98-2	>95	10 mg/m3

UN No: **Not Applicable**
Hazchem Code: **Not Applicable**
DG Class: **Not Applicable**
Subsidiary Risk: **Not Applicable**
Packing Group: **Not Applicable**
Poisons Schedule: **Not Applicable**

HEALTH HAZARD INFORMATION

PRECAUTIONS FOR USE

Appropriate engineering controls:	Local Exhaust Ventilation recommended.
Glasses:	Consider chemical goggles.
Respirator:	Particulate. (AS/NZS 1716 & 1715, EN 143:000 & 149:001, ANSI Z88 or national equivalent)
Storage and Transportation:	Store in cool, dry, protected area. Dispose of this material and its container at hazardous or special waste collection point. Keep out of reach of children.
Fire/Explosion Hazard:	Toxic smoke/fumes in a fire. Dispose of this material and its container at hazardous or special waste collection point.

PROPERTIES

Solid. Does not mix with water.Does not burn.

EMERGENCY

FIRST AID

Swallowed:	Rinse mouth with water.
Eye:	Wash with running water.
Skin:	Wash with soap
Inhaled:	Fresh air. Rest, keep warm. If breathing shallow, give oxygen. Medical attention.
Advice To Doctor:	Treat symptomatically.
Fire Fighting:	Keep surrounding area cool. Water spray/fog.
Spills and Disposal:	Avoid dust. Sweep shovel to safe place. Dispose of this material and its container at hazardous or special waste collection point. This material and its container must be disposed of in a safe way.

SAFE STORAGE WITH OTHER CLASSIFIED CHEMICALS

+	+	+	+	+	+	+

x — *Must not be stored together*
0 — *May be stored together with specific preventions*
+ — *May be stored together*

Appendix 4: Mini MSDS Tetraethyl orthosilicate

Retrieved from the Chemwatch server

HAZARDOUS CHEMICAL. DANGEROUS GOODS. According to the WHS Regulations and the ADG Code.

TETRAETHYL SILICATE

INGREDIENTS	CAS NO	%	8HR OEL
tetraethyl silicate	78-10-4	99.7	85 mg/m3

UN No: **1292**
Hazchem Code: **3Y**
DG Class: **3**
Subsidiary Risk: **Not Applicable**
Packing Group: **III**
Poisons Schedule: **Not Applicable**

HEALTH HAZARD INFORMATION

Acute Health Effects:	Harmful by inhalation. Irritating to eyes. Irritating to respiratory system.

PRECAUTIONS FOR USE

Appropriate engineering controls:	Local Exhaust Ventilation recommended.
Glasses:	Consider chemical goggles.
Respirator:	Type A Filter of sufficient capacity. (AS/NZS 1716 & 1715, EN 143:2000 & 149:2001, ANSI Z88 or national equivalent)
Storage and Transportation:	Store in cool, dry, protected area. Restrictions on Storage apply. Refer to Full Report. Use only in well ventilated areas. Dispose of this material and its container at hazardous or special waste collection point. Keep out of reach of children. Keep away from food, drink and animal feeding stuffs.
Fire/Explosion Hazard:	Flammable. Vapours/gas heavier than air. Toxic smoke/fumes in a fire. Use only in well ventilated areas. Dispose of this material and its container at hazardous or special waste collection point.

PROPERTIES

Liquid.Flammable.

EMERGENCY

FIRST AID

Swallowed:	Rinse mouth with water.
Eye:	Wash with running water.
Skin:	Remove contaminated clothing. Wash with soap & water.
Inhaled:	Fresh air. Rest, keep warm. If breath shallow, give oxygen. Medical attention.
Advice To Doctor:	Treat symptomatically.
Fire Fighting:	Foam.
Spills and Disposal:	Eliminate ignition sources. Prevent from entering drains. Contain spillage by any means. Absorb with dry agent. Stop leak if safe to do so. Use only in well ventilated areas. Dispose of this material and its container at hazardous or special waste collection point. This material and its container must be disposed of in a safe way. To clean the floor and all objects contaminated by this material, use water and detergent.

SAFE STORAGE WITH OTHER CLASSIFIED CHEMICALS

+	x	x	x	+	x	+

x — *Must not be stored together*
0 — *May be stored together with specific preventions*
+ — *May be stored together*

Appendix 5: Mini MSDS Rhodamine 123

Retrieved from the Chemwatch server

NON-HAZARDOUS CHEMICAL. NON-DANGEROUS GOODS. According to the WHS Regulations and the ADG Code.

RHODAMINE 123

INGREDIENTS	CAS NO	%	8HR OEL
rhodamine 123	62669-70-9	>98	-

UN No: **Not Applicable**
Hazchem Code: **Not Applicable**
DG Class: **Not Applicable**
Subsidiary Risk: **Not Applicable**
Packing Group: **Not Applicable**
Poisons Schedule: **Not Applicable**

HEALTH HAZARD INFORMATION

PRECAUTIONS FOR USE

Glasses:	Consider chemical goggles.
Respirator:	Particulate. (AS/NZS 1716 & 1715, EN 143:000 & 149:001, ANSI Z88 or national equivalent)
Storage and Transportation:	Store in cool, dry, protected area.
Fire/Explosion Hazard:	Toxic smoke/fumes in a fire.

PROPERTIES

Solid. Does not mix with water.Does not burn.

EMERGENCY

FIRST AID

Swallowed:	Rinse mouth with water.
Eye:	Wash with running water. For discomfort seek medical advice.
Skin:	Wash with soap
Inhaled:	Blow nose. Rinse mouth with water.
Advice To Doctor:	Treat symptomatically.
Fire Fighting:	Keep surrounding area cool. Water spray/fog.
Spills and Disposal:	Avoid dust. Sweep shovel to safe place.

SAFE STORAGE WITH OTHER CLASSIFIED CHEMICALS

x — *Must not be stored together*
0 — *May be stored together with specific preventions*
+ — *May be stored together*

Appendix 6: Mini MSDS Silicate test

Retrieved from the Chemwatch server, test contains Sulphuric acid.

HAZARDOUS CHEMICAL. DANGEROUS GOODS. According to the WHS Regulations and the ADG Code.

SULFURIC ACID 10% SOLUTION

INGREDIENTS	CAS NO	%	8HR OEL
sulfuric acid	7664-93-9	10	1 mg/m3
water	7732-18-5	90	-

UN No: **3264**
Hazchem Code: **2X**
DG Class: **8**
Subsidiary Risk: **Not Applicable**
Packing Group: **III**
Poisons Schedule: **S6**

HEALTH HAZARD INFORMATION

Acute Health Effects:	Harmful by inhalation. Causes burns. Risk of serious damage to eyes.

PRECAUTIONS FOR USE

Appropriate engineering controls:	General Exhaust Ventilation adequate.
Glasses:	Consider full face-shield.
Gloves:	1.NEOPRENE 2.NATURAL, NEOPRENE
Respirator:	Type E-P Filter of sufficient capacity. (AS/NZS 1716 & 1715, EN 143:2000 & 149:2001, ANSI Z88 or national equivalent)
Storage and Transportation:	Store in cool, dry, protected area. Restrictions on Storage apply. Refer to Full Report. Keep locked up. Keep out of reach of children. Keep away from living quarters. Keep away from food, drink and animal feeding stuffs.
Fire/Explosion Hazard:	Toxic smoke/fumes in a fire. Attacks metals to liberate hydrogen.

PROPERTIES

Liquid.
Mixes with water.
Corrosive.
Acid.Does not burn.

EMERGENCY

FIRST AID

Swallowed:	Give water (if conscious). URGENT MEDICAL ATTENTION.
Eye:	Wash with running water (15 mins). Medical attention.
Skin:	Flood body with water. Remove contaminated clothing. Wash with water
Inhaled:	Fresh air. Rest, keep warm. If breathing shallow, give oxygen. Medical attention.
Advice To Doctor:	Airway problems - 100% O2. Treat burns as thermal. Retract eyelids - irrigate 30 mins. Retract eyelids/ Irrigate 30 mins.
Fire Fighting:	Keep surrounding area cool. Water spray/fog.
Spills and Disposal:	Prevent from entering drains. Contain spillage by any means. Absorb with dry agent. Neutralize with soda ash/ lime. Stop leak if safe to do so. This material and its container must be disposed of in a safe way. To clean the floor and all objects contaminated by this material, use water.

SAFE STORAGE WITH OTHER CLASSIFIED CHEMICALS

+	x	+	0	+	+	+

x — *Must not be stored together*
0 — *May be stored together with specific preventions*
+ — *May be stored together*

Appendix 7: Mini MSDS Ethidium bromide

Retrieved from Thermo Fisher Scientific

GHS Hazard and Precautionary Statements

Hazard Statements: H330-H302-H341

Fatal if inhaled. Harmful if swallowed. Suspected of causing genetic defects.

Precautionary Statements: P260-P284-P201-P281-P304+P340-P320-P330-P310-P405-P501a

Do not breathe dust/fume/gas/mist/vapours/spray. Wear respiratory protection. Obtain special instructions before use. Use personal protective equipment as required. IF INHALED: Remove to fresh air and keep at rest in a position comfortable for breathing. Specific treatment is urgent (see label). Rinse mouth. Immediately call a POISON CENTER or doctor/physician. Store locked up. Dispose of contents/container in accordance with local/regional/national/international regulations.

Other References

Merck	14,4731	**Beilstein**	3642536
Hazard Class	6.1	**Packing Group**	I
Harmonized Tariff Code	2933.99	**TSCA**	No
RTECS	SF7950000		

Appendix 7: Mini MSDS Lithium hexafluorostannate

Retrieved from Thermo Fisher Scientific

GHS Hazard and Precautionary Statements

Hazard Statements: H301-H315-H319-H335

Toxic if swallowed. Causes skin irritation. Causes serious eye irritation. May cause respiratory irritation.

Precautionary Statements: P261-P280-P301+P310-P305+P351+P338-P304+P340-P362-P312-P405-P403+P233-P501a

Avoid breathing dust/fume/gas/mist/vapours/spray. Wear protective gloves/protective clothing/eye protection/face protection. IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF INHALED: Remove to fresh air and keep at rest in a position comfortable for breathing. Take off contaminated clothing and wash before reuse. Call a POISON CENTER or doctor/physician if you feel unwell. Store locked up. Store in a well-ventilated place. Keep container tightly closed. Dispose of contents/container in accordance with local/regional/national/international regulations.

Other References

Hazard Class	6.1	**Packing Group**	III
Harmonized Tariff Code	2826.90	**TSCA**	Yes